Part 2

#11 Deal

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Lloyd Wolfinbarger, Jr.
Appl. No.:08/646,520
Filed: May 7, 1996

Art Unit: 3306
Examiner: Blevis, D.
Atty. Docket: LNRF.011

For: **"A Recirculation Method for Cleaning Essentially Intact Bone Grafts...."**

DECLARATION UNDER RULE 1.131(a)

Commissioner of Patents and Trademarks
Washington, D.C.20231

Dear Sir:

Dr. Lloyd Wolfinbarger, Jr., Phd., the applicant in the above-identified patent application, and Billy G. Anderson, president and CEO of LifeNet Research Foundation, the assignee, declare as follows:

1. That sometime prior to January 21, 1994, Dr. Lloyd Wolfinbarger, a research scientist, conceived of a method for cleaning intact bone grafts using negative or vacuum pressure to remove bone marrow and bone marrow elements from a bone graft as shown in ***Exhibit A***; ***Exhibit A*** attached hereto is a copy of Dr. Wolfinbarger's dated (October 1, 1992) notebook page illustrating the method for cleaning bone using a vacuum mediated flow of solution, the page having three drawings along the left side and a written description describing the drawings along the right side;

2. That the two drawings at the left bottom corner of ***Exhibit A*** establish conception of the present invention because: the middle drawing illustrates a proximal femur where two syringes filled with a detergent solution are inserted into the proximal end of the bone and a vacuum line is attached to the cut end of the bone, arrows are drawn from the syringes pointing downward and continue to and out of the drawn vacuum line indicating that the detergent solution flows from the syringe source through the bone and out the vacuum line by the application of vacuum pressure applied downstream of the vacuum line as indicated by the word "vacuum" written below the drawn vacuum line; and the last drawing illustrates a distal femur where two syringes filled with a detergent solution are inserted into the distal end of the bone and a vacuum line is attached to the cut end of the bone, arrows are drawn from the syringes pointing downward and continue to and out of the drawn vacuum line indicating that the detergent solution flows from the syringe source through the bone and out the vacuum line by the application of vacuum pressure applied downstream of the vacuum line, vacuum pressure as indicated by the word "vacuum" written below the drawn vacuum line;

3. That the syringes illustrated in the drawings in ***Exhibit A*** are filled with a detergent solution as indicated by the written text appearing along the right side of the notebook page, which text under the heading "Objectives" describes using "Panovirocide" which is a solution that includes several detergents, and that the "Panovirocide" is used to "solubilize" the bone marrow tissue, the last paragraph of

text states that the use of "other detergents solutions, i.e. 2% sodium dodecyl sulfate (an anionic detergent) etc." are contemplated; the term "solubilize" describes the action of a detergent and is defined as "to bring into solution any material, especially complexes that normally exist in the cell as part of membrane structures. Such materials usually have hydrophobic domains, and solubilization depends on masking these with detergents or similar substances, to bring the material into micellar suspension." in: *Oxford Dictionary of Biochemistry and Molecular Biology*, edited by Smith, A. D., et al., Oxford University Press, NY (1997);

4. That said method included subjecting the essentially intact bone graft (which is defined in the present specification as including proximal and distal femurs) including the internal matrix thereof to a vacuum mediated flow of solvent to remove bone marrow elements to produce a cell-free bone matrix;

5. That Dr. Lloyd Wolfinbarger successfully performed the method as described in (4) above, prior to January 21, 1994, as evidenced by ***Exhibits B and C***; ***Exhibit B*** is a copy of two pages dated March 1, 1993 showing the results obtained using the vacuum method to clean bone, the graph illustrating protein concentration versus detergent volume; and ***Exhibit C*** is a copy of two photographs, front and back, dated "May 1993" on the back, illustrating bone being processed using the vacuum mediated method;

6. ***Exhibit B***, the graphs, illustrate the amount of protein present in the volume of detergent solution pulled through a bone graft using a vacuum-mediated flow of detergent solution;

7. ***Exhibit C*** is two photographs each showing a distal femur with the articulating distal end immersed in a detergent solution in a beaker and with a vacuum line attached to the cut end of the bone, where the line is attached to a vacuum pump where the pump was operated such that the detergent solution was pulled via vacuum pressure, i.e. a vacuum-mediated flow of detergent solution was induced, through the articulating end of the bone, through the bone, and the detergent solution along with solubilized marrow and debris was drawn to waste;

8. After successful performance of the method in 1993, LifeNet Research Foundation, the assignee of the present application optimized and perfected the method with Dr. Lloyd Wolfinbarger, and filed a patent application on February 27, 1995, issued on September 17, 1996 as U.S. patent no.: 5.556,379 (***Exhibit D***), which is the parent application of the present application, entitled: "Process for Cleaning Large Bone Grafts and Bone Grafts Produced Thereby" which is directed to cleaning bone using a vacuum mediated flow of solution;

9. After filing of the '379 patent application, Standard Operating Procedures (SOP's) were designed, as evidenced by ***Exhibit E*** attached hereto which includes a copy of two documents that are SOP's reflecting the vacuum mediated flow method of cleaning bone, the first is a flow chart and the second is a detailed SOP, that Billy G.

Anderson President and CEO of LifeNet Research Foundation,, put into use on or about May 15, 1995;

10. That Billy G. Anderson President and CEO of LifeNet Research Foundation, on or about May 29, 1996 put the present negative pressure mediated recirculation method as recited in the SOP's attached hereto as ***Exhibit F*** into use as evidenced by ***Exhibit F*** which includes a copy of two documents, which documents show the recirculation method and reflect an effective date of May 29, 1996 and an approval date of May 28, 1996 (document 163.002) and May 21, 1996 (document 034.005);

The declarants further state that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and /or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statement may jeopardize the validity of this application or any patent resulting therefrom.

Mr. Billy G. Anderson, President and CEO

Billy G. Anderson

Date: 3-16-98

Dr. Lloyd Wolfinbarger, Jr., Phd.

L. Wolfinbarger Jr

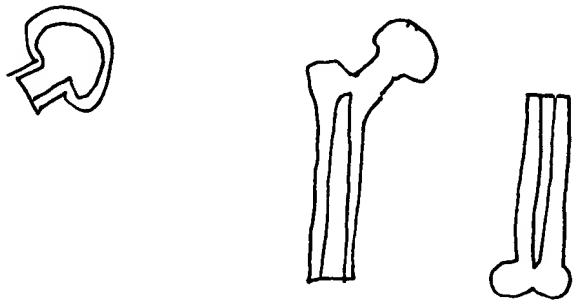
Date: 3/23/98

EXHIBIT A

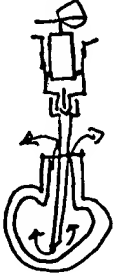
Paraviricide Processing of Bone

10/1 192

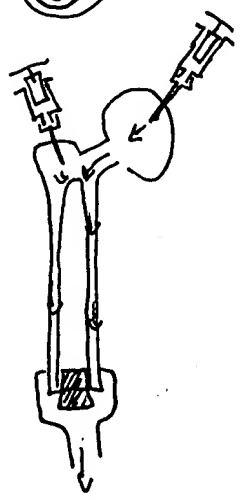
I Focus on Femur heads, proximal femurs, distal femurs.



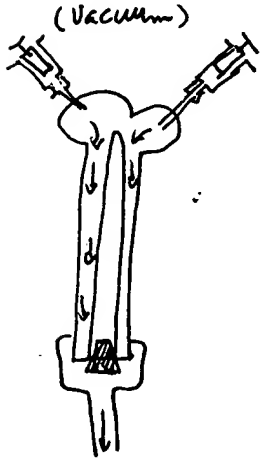
II Flushing protocols



1.) Flush @ varying volumes of paraviricide
2.) Flush @ H_2O_2 (varying volumes)
3.) Flush @ H_2O (varying volumes)
4.) etc.



(Vacuum)



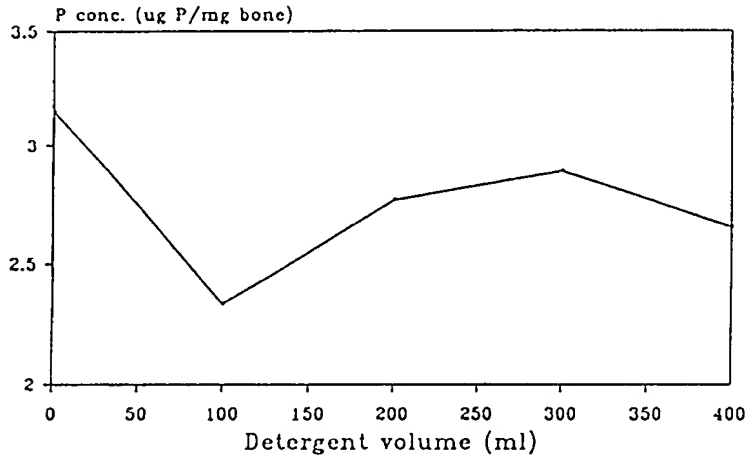
Vacuum

Objective:
— Use Paraviricide to "solubilize" the bone marrow tissue; varying volumes may be necessary to effectively solubilize the bone marrow tissue in the cancellous bone areas.
— use hydrogen peroxide to oxidize remaining tissue debris and create a positive pressure behind the solubilized bone marrow tissue to "force" it out of the cancellous bone.
— use water wash to flush paraviricide & residual H_2O_2 from cancellous bone.
— Options: use other detergent solutions, i.e. 2% sodium dodecyl sulfate (an anionic detergent) etc.

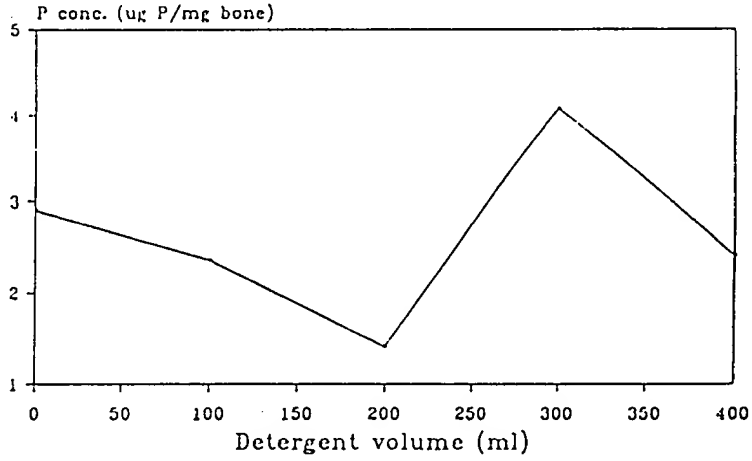
EXHIBIT B

Negative Pressure - Allowash™

Position No.1 – 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



Position No.2 – 3/1/93 Flush
0.5 N NaOH solub., 37 deg., 17 hrs



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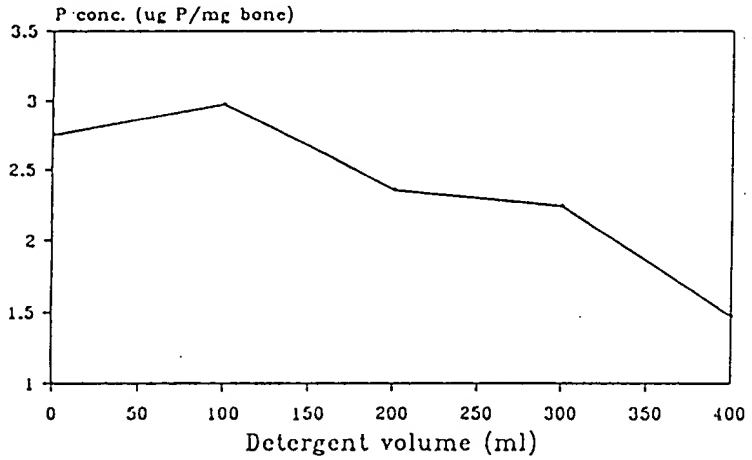
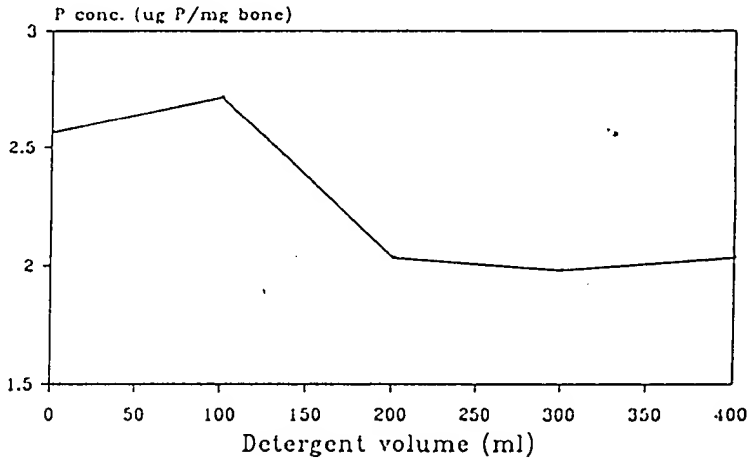
Position No.3 – 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



Position No.4 – 3/1/93 Flus
0.5 N NaOH solub., 37 deg., 17 hrs



Negative Pressure - Allowash™

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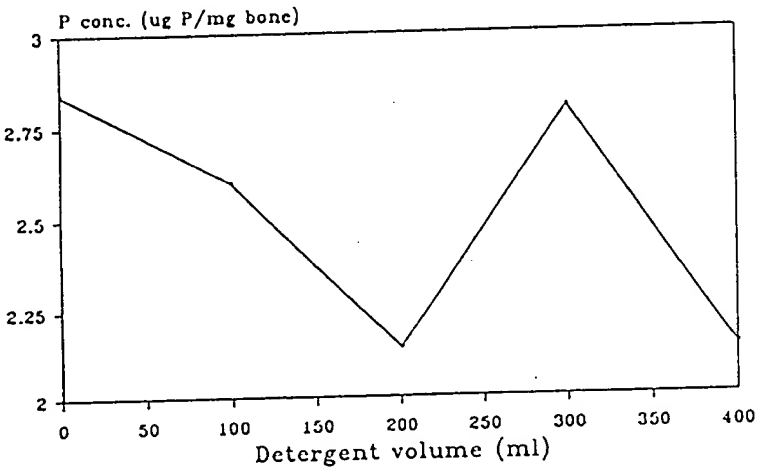
AVERAGE- 3/1/93 Flushing
0.5 N NaOH solub., 37 deg., 17 hrs



AVERAGE- 3/1/93 Flushing
0.5 N NaOH solub., 37 deg., 17 hrs

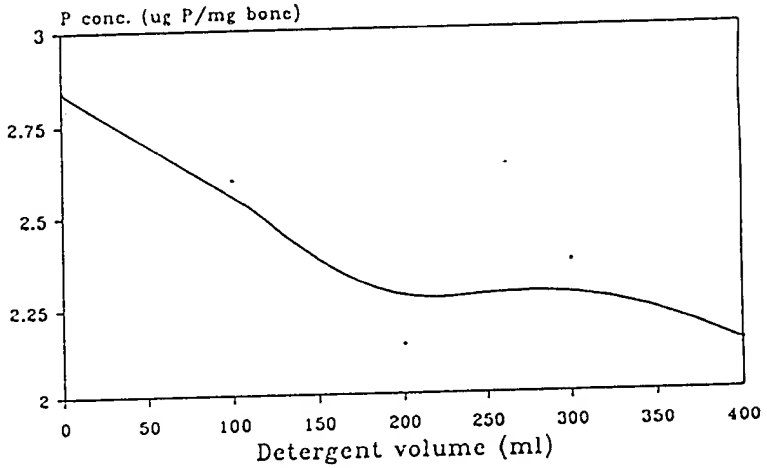
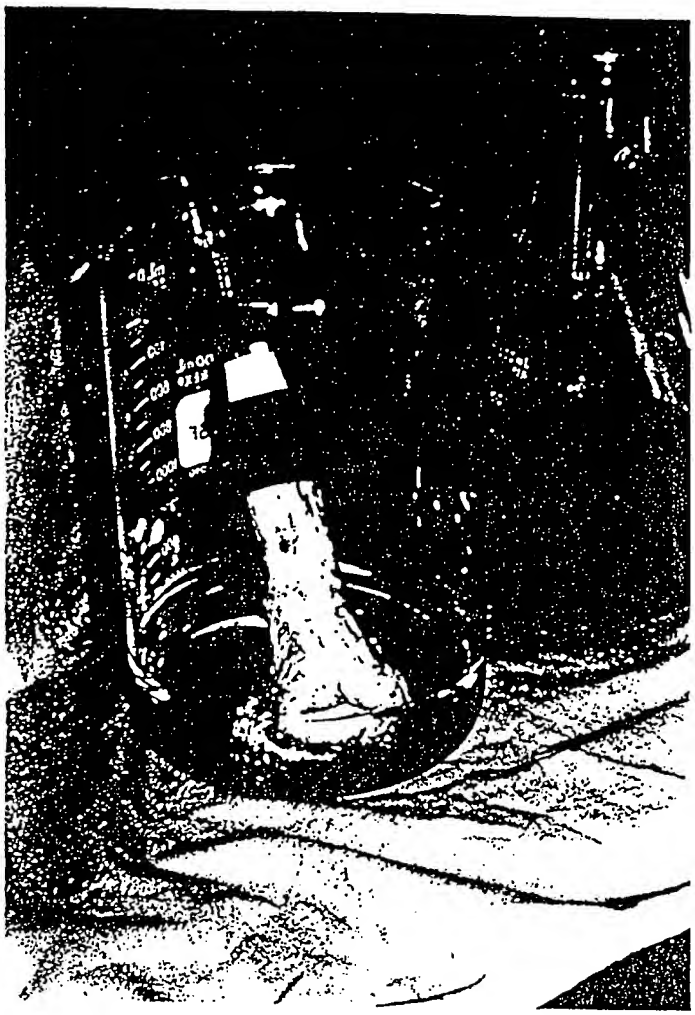


EXHIBIT C



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Allowash™ — Negative Press

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VIRGINIA BEACH, VA 23455

MAY 93

EXHIBIT D

US005556379A

United States Patent [19]

Wolfinbarger

[11] Patent Number: 5,556,379

[45] Date of Patent: Sep. 17, 1996

[54] PROCESS FOR CLEANING LARGE BONE GRAFTS AND BONE GRAFTS PRODUCED THEREBY

[75] Inventor: Lloyd Wolfinbarger, Norfolk, Va.

[73] Assignee: Lifenet Research Foundation, Virginia Beach, Va.

[21] Appl. No.: 395,113

[22] Filed: Feb. 27, 1995

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 293,206, Aug. 19, 1994, abandoned.

[51] Int. Cl.[6] A61M 31/00

[52] U.S. Cl. 604/49; 128/898; 623/16

[58] Field of Search 128/898; 604/28, 604/48, 49; 600/36; 623/16; 435/1, 267, 268

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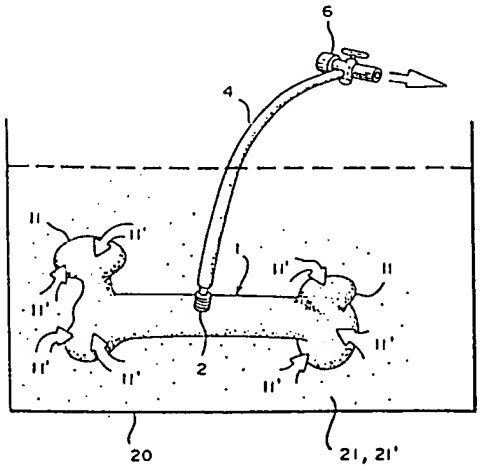
(List continued on next page.)

Primary Examiner—Randall L. Green
Assistant Examiner—V. Alexander
Attorney, Agent, or Firm—Klima & Hopkins, P.C.

[57] ABSTRACT

A process for removing substantially all bone marrow from a large bone and a bone graft produced thereby. A large substantially intact bone is selected and excess cartilage is removed from at least one articulating surface of the bone. An opening is prepared through the cortical layer of the bone to permit access of a vacuum line to the bone cavity. A vacuum line is attached to the bone via the opening for application of vacuum to the bone cavity, and the opening is sealed. A vacuum is applied to draw a first cleaning solution through the bone cavity so as to draw the first solution and solubilized bone marrow through the vacuum line to exit the bone at the opening. The vacuum is discontinued when the bone has been substantially cleaned of bone marrow. Subsequently, a second flushing solution may be drawn through the bone cavity via vacuum.

58 Claims, 6 Drawing Sheets



maintenance fee schedule:

1st - 9.17.2000

2nd - 9.17.2004

3rd - 9.17.2008

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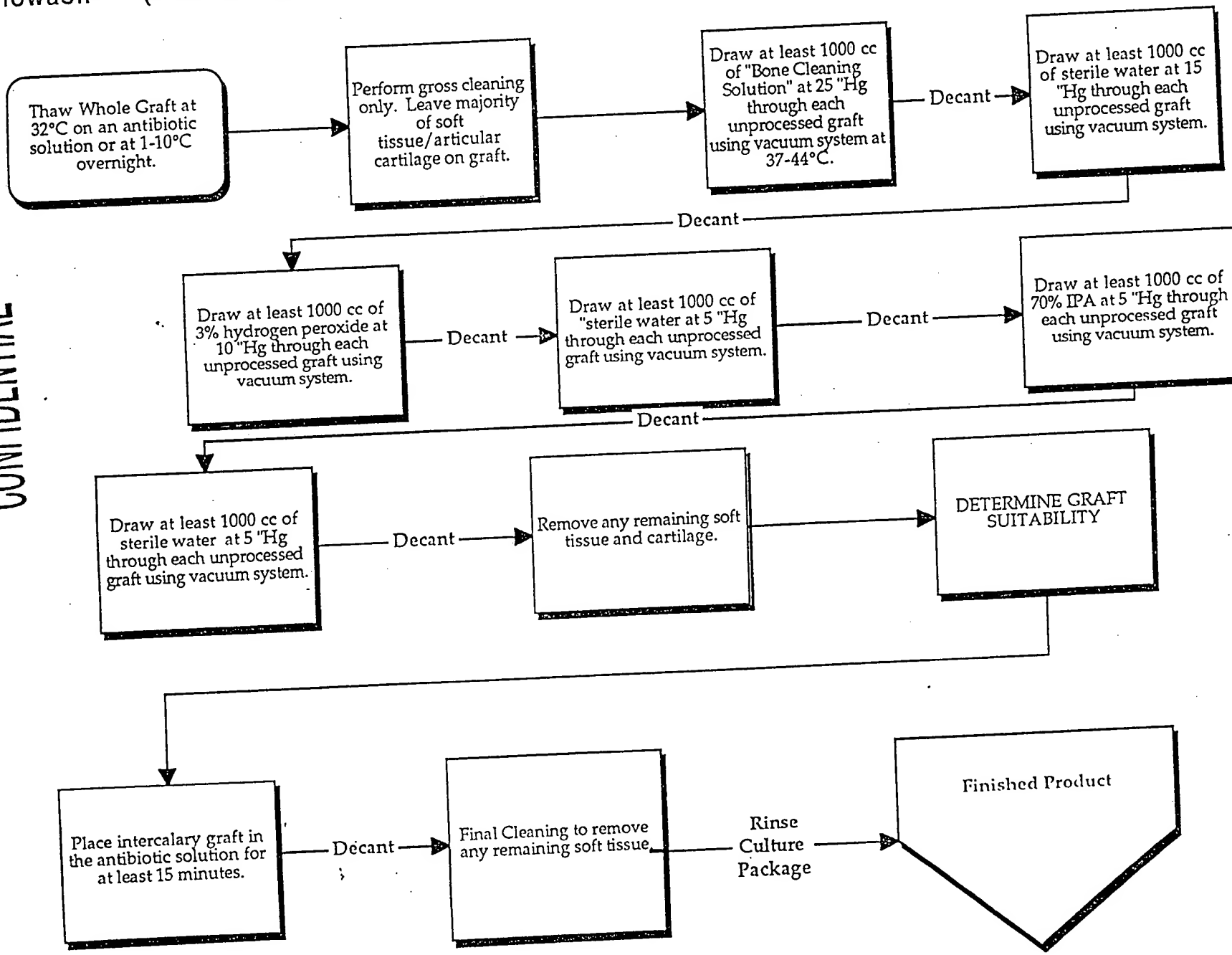
EXHIBIT E

Allowash™ (Vacuum Intercalary Grafts)



Supercedes: PRO-MS-033.002	Written by: BB	Effective Date:
Dept. Approval:	Division Approval:	QA Approval:

Distribution	VP TP Svc	VP Tissue Svcs	VP Corp Svcs	MS	CV	SPD	QA	PTB	SS
Annual QA Review:									

REFERENCES: MUSCULOSKELETAL TISSUE PROCESSING TRAYS, PRO-MS-060, PRO-MS-064

PROCEDURE:

A. Remove some of the soft tissue and periosteum from the graft material using sharp dissection techniques and periosteal elevators. DO NOT REMOVE THE SOFT TISSUE BETWEEN THE FEMORAL HEAD AND GREATER TROCHANTER. THE ENTIRE LIGAMENT MUST BE REMOVED FROM THE FEMORAL HEAD FOVEA.

B. Transect the graft to no more than 22cm in length using a Stryker® saw or band saw. Ensure the cut is straight and contains no bone fragments. Remove all the muscle and periosteum approximately 2" proximal to the cut end.

C. Remove the surface cartilage from the femoral head with either a scalpel blade, elevator, or osteotome.

D. Using a 3/8" drill bit approximately 6" long, remove the contents of the intramedullary canal.

E. Connect the intercalary vacuum apparatus to the cut end of the graft. Wrapping a cut end of the graft with a latex strip prior to attaching the intercalary vacuum apparatus may enhance the connection. Use only enough stripping to ensure a tight seal.

F. Connect an O-clamp to the intercalary apparatus/latex/ and graft junction and tighten. Connect a 1/2" vacuum line to one of the intercalary apparatus. To further enhance the connection, another latex strip and Parafilm® may be wrapped around the junction of the intercalary apparatus and graft.

G. Connect the 1/2" vacuum tubing from the intercalary apparatus to a port on the sterile vacuum manifold. The vacuum manifold is attached to the collection canister at the "Ortho" port using 1/2" vacuum tubing. A 3/16" vacuum line is then attached to the vacuum pump from the collection canister.

Cap all unused ports on the vacuum manifold prior to turning the vacuum pump on.

H. Place the graft and intercalary vacuum apparatus set-up in a deep sterile basin.

I. Pour approximately 6000cc of a 1:100 dilution of Osteoclenz™ into the deep sterile basin. The Osteoclenz™ dilution is prepared by adding 6cc of 10x Osteoclenz™ to 5994 cc of sterile water. Ensure the basin is clearly labeled as "Osteoclenz™."

The temperature of the solution must be between 37° and 44° C. Place the deep sterile pan on a warmer if necessary. Each of the grafts must remain immersed in fluids at all times.

J. Critical Step: Set the vacuum pressure to 25 in.Hg and draw the Osteoclenz™ through the graft(s). At least 1L of Osteoclenz™ must be infused through each allograft. Record the vacuum pressure, volume of infused solution and temperature of the Osteoclenz™ on the "Tissue Processing Log Worksheet."

Periodically inspect the vacuum lines. Any large bubbles indicate a leak in the vacuum tubing set-up. Tighten the O-Ring, reapply the latex stripping , or add Parafilm® to secure the seal.

K. Critical Step: Decant any remaining 1:100 dilution of Osteoclenz™ solution and add approximately 6L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of water through the graft at 15 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

L. Critical Step: Decant any remaining sterile water and add approximately 6L of 3% hydrogen peroxide to the deep sterile basin. Ensure the basin is clearly labeled as "Hydrogen Peroxide." Draw at least 1L of 3% hydrogen peroxide through the graft at 10 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

M. Critical Step.: Decant any remaining 3% hydrogen peroxide and add approximately 6L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of sterile water through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

N. Decant any remaining sterile water and add approximately 6L of 70% isopropyl alcohol to the deep sterile basin. Ensure the basin is clearly labeled as "70% IPA." Draw at least 1L of 70% isoptopyl alcohol through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

O. Critical Step.: Decant any remaining 70% isopropyl alcohol and add 3L of sterile water to the deep sterile basin. Ensure the basin is clearly labeled as "Sterile Water." Draw at least 1L of sterile water through the graft at 5 in.Hg. Record the vacuum pressure, volume of infused solution and exposure time on the "Tissue Processing Log Worksheet."

P. Remove the remaining soft tissue and cartilage and place the graft(s) in a basin with the antibiotic solution for at least 15 minutes. Ensure the basin is clearly labeled as "Antibiotics." Record the exposure time to the antibiotics on the "Tissue Processing Log Worksheet."

Q. Remove any remaining soft tissue if necessary and rinse again before culturing.

If the sterile wire wheel is used to remove the remaining soft tissue, ensure the Lucite™ capture box is surrounding the graft to minimize soft tissue discharge.

R. Assign the graft(s) the appropriate identification number, record the measurements on the processing log worksheet (record the condyle width, side, and total graft length), and culture the fashioned graft(s) for bacterial contamination with sterile cotton-tipped applicators. Place one cotton-tipped applicator into a TGC tube, and one into a TSB tube, label with the graft identification number. Refer to PRO-MS-060.

S. The graft material is now ready for packaging and placement in the appropriate freezer. Refer toPRO-MS-064. The graft must be x-rayed if requested.

Allowash™ Treated Frozen Intercalary Tissue/Positive Pressure

Frozen Proximal Tibia
Frozen Proximal Humerus
Frozen Proximal Femur with Head
Frozen Proximal Femur
Frozen Distal Femur

EXHIBIT F

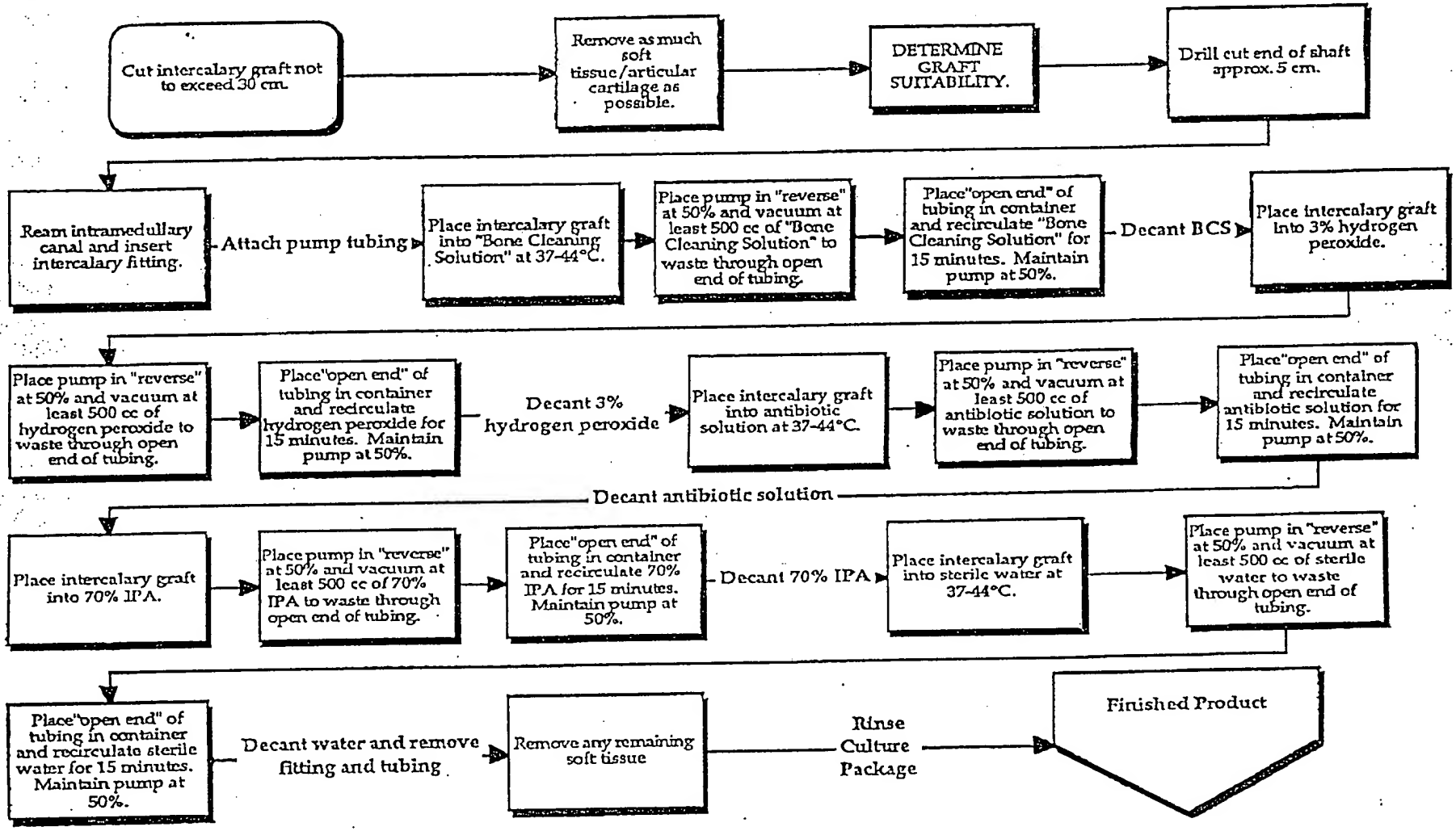
CONFIDENTIAL

DOCUMENT#: PRO-MS-163.002

TITLE: ALLOWASH™ INTERCALARY GRAFT FLOW DIAGRAM

Superceded: PRO-MS-163.001	Written by: BB	Effective Date: MAY 29 1996
Dept. Approval: BB 5/28/96	Division Approval: 5/28/96	QA Approval:
Distribution: VP TP Svcs VP Tissue Svcs VP Corp Svcs MS CV SPD QA SS		
Annual QA Review:		

REFERENCES: PRO-MS-036, PRO-MS-035, PRO-MS-034, PRO-MS-033, PRO-MS-028



Supercedes: PRO-MS-034.004	Written by: CKW,AH	Effective Date: ~~May 29, 1996~~ 1996 (5/28/96)
Dept. Approval: 5/21/96	Division Approval: 5/23/96	QA Approval: 5/23/96

Distribution:	VP TP Svc	VP Tissue Svcs	VP Corp Svcs	MS	CV	SPD	QA	SS
Annual QA Review:								

REFERENCES: PRO-MS-010, PRO-MS-060, PRO-MS-064, PRO-MS-136

PROCEDURE:

A. Transect the graft to the desired length using a Stryker® saw or band saw. Ensure each bisected piece is not more than 30 cm in length. Ensure the cut is straight and contains no bone fragments.

B. Remove all of the soft tissue and periosteum from the graft material using sharp dissection techniques and periosteal elevators.

C. Remove the surface cartilage from the femoral head with either a scalpel blade, periosteal elevator, or osteotome. The processing instructions may dictate leaving the cartilage "on" when appropriate.

D. Using a 3/8" drill bit, drill the cut end of the shaft approximately 5 cm. Thoroughly wash the interior of the intramedullary canal with the lavage system.

E. Insert the intercalary fitting by screwing the threaded, tapered end into the cut end of the graft.

F. Assemble the vacuum tubing by securing one end of the tubing to the nipple end of the intercalary fitting. Secure the other end of the tubing to the piston driven pump. Finally, secure another section of vacuum tubing to the other side of the piston pump.

G. Pour approximately 4000 cc of a 1:100 dilution of the "Bone Cleaning Solution" into the sterile flushing vessel. The "Bone Cleaning Solution" is prepared by adding 4 cc of cleaning reagent to 3996 cc of sterile water. Refer to PRO-MS-136. Ensure the flushing vessel is clearly labeled as "Bone Cleaning Solution".

The temperature of the "Bone Cleaning Solution" solution must be between 37 ° and 44°C.

H. Critical step: Place the "open" end of the second piece of vacuum tubing into a graduated Fleaker™ flask. Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of the "Bone Cleaning Solution" to waste. Record the vacuum times, the approximate volume of solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

I. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the "Bone Cleaning Solution" to recirculate for a minimum of 15 minutes. Record the temperature, exposure time, and flow rate controller setting on the "Tissue Processing Log Worksheet."

J. Critical step: Decant the 1:100 dilution of the "Bone Cleaning Solution" and add approximately 4L of 3% hydrogen peroxide to the flushing vessel. Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of the 3% hydrogen peroxide solution to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

K. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the flow rate controller in the "reverse" position at 50%. Allow the hydrogen peroxide to recirculate for a minimum of 15 minutes. Ensure the flushing vessel is clearly labeled as "3% Hydrogen Peroxide". Record the exposure time and the flow rate controller setting on the "Tissue Processing Log Worksheet."

L. Critical step: Decant the hydrogen peroxide and add approximately 3980 cc of sterile water and the entire reconstituted vials of Bacitracin and Polymyxin B to the flushing vessel. Refer to PRO-MS-010. Ensure the flushing vessel is clearly labeled as "Antibiotic." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of antibiotic solution to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

The temperature of the water/antibiotic solution must be between 37° and 44° C.

M. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the antibiotic solution to recirculate for a minimum of 15 minutes. Record the temperature of the antibiotic solution, exposure time, and flow rate controller setting on the "Tissue Processing Log Worksheet."

N. Critical step: Decant the antibiotic solution and add approximately 4L of 70% isopropyl alcohol to the flushing vessel. Ensure the flushing vessel is clearly labeled as "70% IPA." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of 70% IPA to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

O. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the IPA to recirculate for a minimum of 15 minutes. Record the exposure time and the flow rate controller setting on the "Tissue Processing Log Worksheet."

P. Critical step: Decant the 70% isopropyl alcohol and add 4L of sterile water to the flushing vessel. Ensure the flushing vessel is clearly labeled as "Sterile Water." Set the piston pump to "reverse" and set the flow rate controller to 50%. Turn on the pump and vacuum at least 500 cc of sterile water to waste. Record vacuum time, approximate volume of discarded solution, and the flow rate controller setting on the "Tissue Processing Log Worksheet". Do not allow the level of fluid in the column to fall below the intercalary fitting.

The temperature of the sterile water must be between 37° and 41° C.

Q. Critical step: Remove the "open" end of the second piece of vacuum tubing from the graduated Fleaker™ flask and place it into the sterile flushing vessel. Maintain the drive in the "reverse" position at 50%. Allow the water to recirculate for a minimum of 15 minutes. Record the temperature of the water, exposure time, and the flow rate controller setting on the "Tissue Processing Log Worksheet."

R. Disconnect the tubing and remove the intercalary fitting from the graft.

S. Remove any remaining soft tissue if necessary and rinse again before culturing.

If the sterile wire wheel is used to remove the remaining soft tissue, ensure the Lucite™ capture box is surrounding the graft to minimize soft tissue discharge.

T. Assign the graft the appropriate identification number, record the measurements on the processing log worksheet (record the side (R/L), femoral head size, and total graft length). Culture the fashioned graft for bacterial contamination with sterile cotton-tipped applicators. Place one cotton-tipped applicator into a TGC tube, and one into a TSB tube, label with the graft identification number. Refer to PRO-MS-060.

U. The graft material is now ready for packaging and placement in the appropriate freezer. Refer to PRO-MS-064. The graft must be x-rayed if requested.